

Development of sprouted wheat based probiotic beverage

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Abstract Present study was carried out to evaluate the potential of *Lactobacillus acidophilus* (*L. acidophilus*) for development of wheat based probiotic beverage and to optimize the proportion of different ingredients viz. sprouted wheat flour, sprouted wheat bran, oat and stabilizer using response surface methodology. Acidity, pH and probiotic count of samples prepared with *L. acidophilus* NCDC-14 was higher than that of *L. acidophilus* NCDC-16 culture. Being more compatible, *L. acidophilus* NCDC-14 was selected for this study. Acidity (in terms of lactic acid), pH and probiotic count of the different samples ranged from 0.21 to 0.45 %, 4.0 to 4.9, and 8.30 to 10.95 \log_{10} cfumL⁻¹, respectively. Probiotic count increased with increasing amount of sprouted wheat and oat. Optimized levels for sprouted wheat flour, oat, wheat bran and guar gum were 7.86, 5.42, 1.42 and 0.6 g, respectively per 100 mL of water. Optimized probiotic beverage provided 13.19 % total solids, 1.19 % protein, 0.33 % fat, 0.10 % ash, 0.42 % crude fibre, 1.45 mg iron, calcium 15.74 mg, 11.56 % carbohydrates, 54 kcal calories and 10.43 \log_{10} cfumL⁻¹ probiotic count. Thus, *Lactobacillus acidophilus* NCDC-14 can be used for development of potentially probiotic beverage with sprouted wheat and oat.

Keywords *Lactobacillus acidophilus*-NCDC14 · Sprouted wheat · Probiotic beverage

Introduction

Tremendous changes in lifestyle, eating habits and shifting rural habitations are causing an irreversible change that is leading to manifold multiplication of health problems. Due

to huge expenditure on health care each year, consumers' desire for food products with desired health benefits continues to grow. Consumers are interested in foods that boost the immune system, reduce the risk of disease and enhance health, which consumers self-prescribe for themselves and their families. Nowadays functional foods are gaining public acceptance in many countries. The market surveys showed that there is great scope for value-added as well as health promoting food products (Singh 2007).

A major development in functional foods pertains to foods containing probiotics and prebiotics which enhance health promoting microbial flora in the intestine. There is growing scientific evidence to support the concept that live microorganisms when administered in adequate amounts confer a health benefit on the host by improving its intestinal microbial balance (FAO/WHO 2001; Holzapfel et al. 2001; Fuller 1992). A number of genera of bacteria (and yeast) are used as probiotics, including *Lactobacillus*, *Leuconostoc*, *Pediococcus*, *Bifidobacterium*, and *Enterococcus*, but the main species believed to have probiotic characteristics are *L. acidophilus*, *Bifidobacterium* spp., and *L. casei* (Sharma and Mridula 2013). Considering the different intestinal bacterial groups, it is well known that bifidobacteria and lactobacilli can be used as probiotics, i.e. live microbial food ingredients that are beneficial to health. Various efforts have been made in order to increase in the colon the number and/or the activity of the bacterial groups considered beneficial for the host and to decrease those considered as harmful (Matteuzia et al. 2004). By increasing the amount of prebiotics in the diet, it is possible to increase and maintain healthy bacterial gut flora in the host (Gibson et al. 2003). Ingredients in certain food products may naturally contain prebiotics which help to improve the functional efficacy of probiotics. Foods can also be fortified with prebiotics during manufacturing process to increase probiotic efficacy (Ranadheera et al. 2010). When both prebiotics and probiotics are present in a food then those functional foods are referred to as synbiotic (Pandiyani et al. 2012).

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Foods used for dissemination of probiotics are usually fermented foods however, probiotics could also be present in infant formula, fruit drinks, whey drinks and sweet milk. Probiotic LAB (Lactic acid bacteria), especially *Lactobacillus* and *Bifidobacterium*, are known to enhance the capacity of host to fight against intestinal infections by stimulating the mucosal immune system (Erickson and Hubbard 2000). *Lactobacillus acidophilus* strains are widely used as probiotic cultures in dairy products because this species possess therapeutic properties (Pandiyani et al. 2012). Due to the proven health-promoting effects, the lactobacilli have commonly been marketed as probiotics (Shah 2000; Tannock 2004; Bernardeau et al. 2006; Saran et al. 2012). In recent years, cereals have also been investigated regarding their potential use in developing functional foods. Lactic acid fermentation of cereals is a long-established processing method and is being used in Asia and Africa for the production of foods in various forms such as beverages, gruels, and porridge. Cereals contain water-soluble fiber (such as β -glucan and arabinoxylan), oligosaccharides (such as galacto- and fructo oligosaccharides) and resistant starch, and thus have been suggested to fulfill the prebiotic concept (Shah 2001). Whole grains are also sources of many phytochemicals, including phytoestrogens, phenolic compounds, antioxidants, phytic acid and sterols. Lactic acid fermentation usually improves the nutritional value and digestibility of cereals (Charalampopoulos et al. 2002). Lactic acid fermentation of different cereals, such as maize, sorghum, finger millet, has been found effective in reducing the amount of phytic acid, tannins and improve protein digestibility (Chavan et al. 1988; Lorri and Svanberg 1993). In a food product, concentration of approximately 10^7 probiotic bacterial cells/ml at the time of consumption is considered functional (Gomes and Malcata 1999; Shortt 1999). The *Lactobacillus acidophilus* strains have been used as probiotic bacteria in various food formulations such as yoghurt, curd, ice cream (Bajad et al. 2006; Yadav et al. 2007; Jain et al. 2008; Pandiyani et al. 2012). Arora et al. (2010) had also developed barley based probiotic food mixture using *Lactobacillus acidophilus* and recommended a combination of germination and fermentation as a potential process for enhancing the nutritional quality of cereal based food mixes. Freeze dried cultures of *Lactobacillus acidophilus* NCDC 14, has proven therapeutic benefits (Reddy et al. 2006; Singh et al. 2007).

The objective of this study was to evaluate the potential of *L. acidophilus*- NCDC14 for development of wheat based probiotic beverage (WPB) and to optimize the proportion of different ingredients viz. sprouted wheat flour, sprouted wheat bran, oat and stabilizer i.e. guar gum with the aim of maximizing probiotic count for preparation of probiotic beverage.

Material & methods

Raw materials

Wheat (cv.PBW550) were cleaned, washed and soaked in water in the ratio of 1:2 (seeds to water) for 8 h at room temperature. After draining the water, the grains were allowed to sprout at controlled temperature (35 °C) and 95 % Relative Humidity. The sprouted wheat was dried at 50 °C in a cabinet tray dryer to 8.86 % moisture level. The rootlets of sprouted and dried wheat were removed by hand scrubbing. Wheat bran was obtained by pearling sprouted wheat for 1 min using grain pearler (Make: CIAE Bhopal, 100–300 kg/h). Oat (Quaker oats) and Guar Gum (SD Fine Chem. Ltd.) were procured from local market. Finally, sprouted wheat, bran and oat were ground and sieved through 85 mesh sieve to obtain a fine formulation (particle size 0.177 mm).

Probiotic cultures

Two probiotic strains namely *L. acidophilus* NCDC 14 and *L. acidophilus* NCDC 16 were procured from National Collection of Dairy Cultures, NDRI, Karnal, Haryana, India. The strains were maintained at 4 °C and sub cultured monthly on slants prepared from MRS (de Man Rogosa Sharpe) agar.

Activation of microbial culture and extraction of pellet

Culture was activated in MRS broth by transferring 0.1 mg of freeze dried culture in 10 ml of MRS broth and the tube was incubated at 37 °C for 24 to 48 h. From this 10 ml, 1 ml was taken in 100 mL MRS broth and this culture was reactivated at 37 °C for 24–48 h with several transfers (6 times in 10 mL) of the culture. At last 1 mL from the last 10 mL was taken in 100 mL MRS Broth and incubated at 37 °C for 24–48 h. 1 mL of the activated culture was placed on MRS Agar at 37 °C for 48 h. After 48 h, colonies were picked and gram staining was done for checking the purity of the culture. Rod shaped pink coloured colonies were observed under microscopic and these were picked and their growth was observed in MRS broth at 37 °C for 24–48 h.

Extraction of microbial cell pellets

The activated culture was centrifuged in sterilized centrifuge bottles at 4,500 rpm for 10 min at 4 °C using a bench top refrigerated centrifuge. After centrifugation, supernatant portion of the tube was decanted and microbial pellets were washed in sterilized bottle using sterilized 25–30 ml deionized water/peptone water and centrifuged. The washed pellets were re-centrifuged at 4,500 rpm for 10 min at 4°C to remove traces of MRS broth. The cell concentration was tested using pour

Table 1 Box-Behnken design with values of independent and dependent variables of WPB

Experiments	Factor 1 Sprouted wheat flour g/100 ml	Factor 2 Oat g/100 ml	Factor 3 Sprouted wheat bran g/100 ml	Factor 4 Guar gum g/100 ml	Response 1 pH	Response 2 Acidity (% in lactic acid)	Response 3 Probiotic count (log ₁₀ cfu mL ⁻¹)
1	6	2	2	0.2	4.4	0.37	8.30
2	6	6	1	0.4	4.1	0.45	10.30
3	8	4	3	0.4	4.4	0.39	9.48
4	8	4	2	0.6	4.7	0.225	9.78
5	6	2	3	0.4	4.29	0.41	8.48
6	6	4	3	0.6	4.4	0.38	8.78
7	4	4	2	0.6	4.6	0.27	8.85
8	6	4	3	0.2	4.2	0.405	8.95
9	8	6	2	0.4	4	0.45	10.70
10	4	4	1	0.4	4.9	0.315	9.70
11	6	4	1	0.6	4.2	0.425	9.60
12	6	4	2	0.4	4.4	0.375	9.70
13	8	2	2	0.4	4.51	0.225	8.85
14	6	2	1	0.4	4.3	0.405	8.70
15	4	4	3	0.4	4.7	0.32	9.60
16	6	4	2	0.4	4.77	0.38	8.48
17	8	4	1	0.4	4.1	0.435	10.48
18	6	6	3	0.4	4.4	0.405	10.95
19	6	6	2	0.2	4.6	0.45	10.78
20	4	2	2	0.4	4.54	0.225	8.78
21	6	4	2	0.4	4.3	0.225	8.60
22	6	4	2	0.4	4.7	0.21	9.90
23	6	4	1	0.2	4.4	0.36	10.30
24	6	4	2	0.4	4.8	0.27	8.60
25	4	6	2	0.4	4.27	0.36	10.85
26	6	2	2	0.6	4.71	0.28	8.70
27	4	4	2	0.2	4.81	0.25	8.85
28	6	6	2	0.6	4.1	0.45	10.70
29	8	4	2	0.2	4.27	0.415	8.78

WPB wheat based probiotic beverage; Values of responses are means of three replicates

plate technique and then adjusted to ~10 log₁₀CFU per mL by suspending in 0.1 % peptone solution (w/v).

Selection of probiotic bacteria

Sprouted wheat flour mixture (10 g per 100 mL), prepared in sterile water was heated to 90 °C and hold for 5 min. After cooling the flour mixture at room temperature (about 30–35 °C), 1 % (v/v) probiotic culture (probiotic count adjusted to ~8 log₁₀CFU per mL) was added and incubated for at 37 °C. Both *L. acidophilus* NCDC 14 and *L. acidophilus* NCDC 16 were added separately to these wheat flour slurries. Control samples without any wheat flour (ie. Only containing sterile water and probiotic culture) were also prepared in the similar manner.

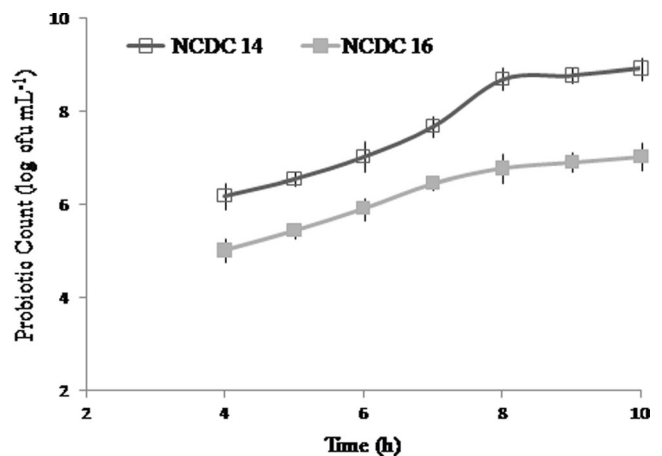


Fig. 1 Probiotic count of *Lactobacillus acidophilus* NCDC 14 and NCDC 16 incorporated sprouted wheat slurry at different time intervals

Table 2 Analysis of variance for pH of WPB using response surface quadratic model

Source of variations	SS	df	MS	F value	P value
Model	1.195404	14	0.085386	2.309164	0.0647 ^{ns}
A	0.282133	1	0.282133	7.629963	0.0153 [*]
B	0.136533	1	0.136533	3.692383	0.0753 ^{ns}
C	0.012675	1	0.012675	0.34278	0.5675 ^{ns}
D	7.5E-05	1	7.5E-05	0.002028	0.9647 ^{ns}
AB	0.0144	1	0.0144	0.389431	0.5426 ^{ns}
AC	0.0625	1	0.0625	1.690239	0.2146 ^{ns}
AD	0.1024	1	0.1024	2.769287	0.1183 ^{ns}
BC	0.024025	1	0.024025	0.649728	0.4337 ^{ns}
BD	0.164025	1	0.164025	4.435863	0.0537 ^{ns}
CD	0.04	1	0.04	1.081753	0.3159 ^{ns}
A ²	0.001558	1	0.001558	0.042145	0.8403 ^{ns}
B ²	0.214858	1	0.214858	5.810591	0.0303 [*]
C ²	0.167614	1	0.167614	4.532935	0.0515 ^{ns}
D ²	0.00829	1	0.00829	0.224197	0.6432 ^{ns}
Residual	0.517678	14	0.036977		
Lack of Fit	0.308958	10	0.030896	0.592101	0.7716 ^{ns}
Pure Error	0.20872	4	0.05218		
Correlation Total	1.713083	28			

WPB wheat based probiotic beverage; SS- sum of squares; df- degree of freedom; MS- mean sum of squares; A- sprouted wheat; B- oat; C- sprouted wheat bran; D- guar gum, **p*<0.05; ns- non significant

Enumeration of probiotic count was done after 4, 5, 6, 7, 8, 9 and 10 h by the pour plate method as described by Shah (2000) using MRS agar. Enumeration of probiotic count was done Sterile peptone solution (0.1 %w/v) was used for making dilutions of samples.

Experimental design

The amount of sprouted wheat flour, oat, sprouted wheat bran and stabilizer (guar gum) were optimized using the Box-Behnken design of response surface methodology

Table 3 Analysis of variance for acidity of WPB using re- sponse surface quadratic model

Source of variations	SS	df	MS	F value	P value
Model	0.118353	14	0.008454	3.569001	0.0117 [*]
A	0.045019	1	0.045019	19.00599	0.0007 ^{**}
B	0.018408	1	0.018408	7.771621	0.0145 [*]
C	8.33E-06	1	8.33E-06	0.003518	0.9535
D	0.000102	1	0.000102	0.043097	0.8385
AB	0.001056	1	0.001056	0.445927	0.5151
AC	0.000625	1	0.000625	0.263862	0.6155
AD	0.001225	1	0.001225	0.51717	0.4839
BC	0.0049	1	0.0049	2.06868	0.1723
BD	0.000156	1	0.000156	0.065966	0.8010
CD	0.002025	1	0.002025	0.854913	0.3708
A ²	0.000139	1	0.000139	0.058577	0.8123
B ²	0.023546	1	0.023546	9.940787	0.0071 ^{**}
C ²	0.018915	1	0.018915	7.985354	0.0135 [*]
D ²	0.015974	1	0.015974	6.743847	0.0211 [*]
Residual	0.033161	14	0.002369		
Lack of Fit	0.006831	10	0.000683	0.103779	0.9982 ^{ns}
Pure Error	0.02633	4	0.006583		
Correlation Total	0.151514	28			

WPB wheat based probiotic beverage; SS- sum of squares; df- degree of freedom; MS- mean sum of squares; A- sprouted wheat; B- oat; C- sprouted wheat bran; D- guar gum, **p*<0.05; ***p*<0.01; ns- non significant

(RSM). This design was preferred, as it is made to require only 3 levels, coded as -1 , 0 , and $+1$. Box-Behnken designs are available for 3 to 21 factors. They are formed by combining two-level factorial designs with incomplete block designs. This procedure creates designs with desirable statistical properties but, most importantly, with only a fraction of the experiments required for a three-level factorial. The levels of different independent variables and plan of experiment for the present study are given in Table 1. Based upon the statistical analysis of the data, three optimized formulations were selected with desirability level >0.87 for validation purposes (Table 6).

Process for preparation of wheat based probiotic beverage

The level of different ingredients as obtained from the experimental design were mixed in sterile water in the laminar air flow in order to avoid contamination and covered tightly using sterile caps. The mixture was heated to $90\text{ }^{\circ}\text{C}$ for 5 min and cooled to room temperature (about $30\text{--}35\text{ }^{\circ}\text{C}$). 1 % (v/v) *L. acidophilus* NCDC 14 probiotic culture

(probiotic count adjusted to $\sim 10\text{ log}_{10}$ cfu per mL) was added to this flour mixture and incubated for at $37\text{ }^{\circ}\text{C}$ for 8 h. All the experiments were conducted in triplicates.

Proximate composition

Moisture, protein (using the factor $6.5 \times N$), crude fat, ash, crude fibre, calcium and iron in probiotic samples were determined as per standard methods (AOAC 2000). Total carbohydrate value was obtained by difference. Total calories were calculated by multiplying protein, carbohydrates and fat content by 4, 4 and 9, respectively. All the chemicals used for estimation of proximate composition were of AR grade.

Data analysis

The data were analyzed for three replicates using Design Expert 7.0 software for designing and analyzing of response surface data. This software also optimized the independent variables based on conditions.

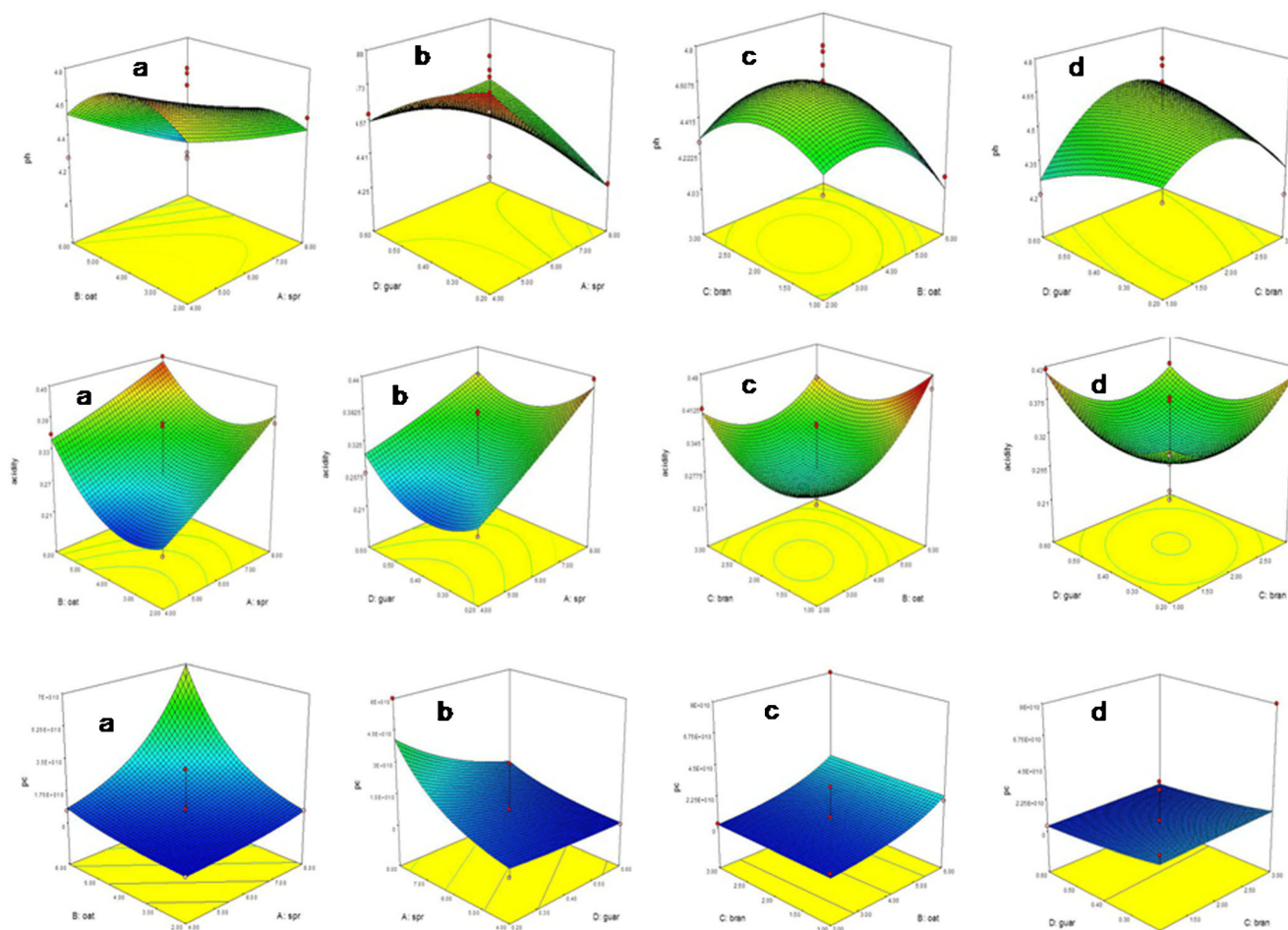


Fig. 2 Response surface curves for combined effect of (a) oat and sprouted wheat flour, (b) guar gum and sprouted wheat flour, (c) bran and oat, (d) guar gum and bran on pH, acidity and probiotic count of probiotic beverage

Table 4 Analysis of variance for probiotic count of WPB using response surface linear model

Source of variations	SS	df	MS	F value	P value
Model	32.74519	4	8.186298	6.341315	0.0012**
A	13.07585	1	13.07585	10.12889	0.0040**
B	15.25018	1	15.25018	11.81318	0.0022**
C	0.011848	1	0.011848	0.009178	0.9245 ^{ns}
D	4.407315	1	4.407315	3.414018	0.0770 ^{ns}
Residual	30.98272	24	1.290946		
Lack of Fit	21.16977	20	1.058488	0.431466	0.9076 ^{ns}
Pure Error	9.812949	4	2.453237		
Correlation Total	63.72791	28			

WPB wheat based probiotic beverage; SS- sum of squares; df- degree of freedom; MS- mean sum of squares; A- sprouted wheat; B-oat; C- sprouted wheat bran; D-guar gum, ***p*<0.01; ns- non significant

Results & discussions

Selection of probiotic bacteria

The probiotic count was enumerated for the sprouted wheat slurry prepared using *L. acidophilus* NCDC 14 and *L. acidophilus* NCDC 16 probiotic cultures. The probiotic count was higher in the wheat slurry containing *L. acidophilus* NCDC 14 and the count was fairly good after 8 h of incubation at 37 °C (Fig. 1). Thus, the time period selected for the development of wheat based probiotic beverage was 8 h. The values for pH, acidity and probiotic count of the samples prepared with *L. acidophilus* NCDC-14 and *L. acidophilus* NCDC-16 culture were 5.08 and 5.21, 0.0352 % and 0.0285 % and 8.70 log₁₀ cfumL⁻¹ and 6.78 log₁₀ cfumL⁻¹, respectively. Thus, from these experiments; the better compatible strain, i.e. *L. acidophilus* NCDC-14 was selected for further study.

Fitting the models

The observed values of all the dependent variables along with the level of independent variables for all the probiotic beverage samples are given in Table 1. The pH values for different samples ranged from 4.0 to 4.9, while the acidity of the probiotic beverage samples varied from 0.21 to 0.45 %.

The probiotic counts of the different samples were in the range of 8.30 to 10.95 log₁₀ cfumL⁻¹.

Effect of ingredients' level on pH Analysis of variance for the response surface quadratic model for pH is given in Table 2. The F value of 2.309 and p value greater than 0.05 indicate that the model for pH is not significant. It is evident from the results that there is no significant effect of amount of sprouted wheat bran, oat and guar gum on pH values of the probiotic beverage, however, significant effect has been observed by the level of sprouted wheat flour. The response surface graphs for the combined effect of levels of different ingredients on pH are given in 2. The second order polynomial model was obtained by model fitting for pH as the function of sprouted wheat flour, sprouted wheat bran, oat and guar gum as:

$$\begin{aligned}
 \text{pH} = & 4.594 - 0.153333333 * A - 0.106666667 * B \\
 & + 0.0325 * C + 0.0025 * D - 0.06 * A * B \\
 & + 0.125 * A * C + 0.16 * A * D + 0.0775 * B * C \\
 & - 0.2025 * B * D + 0.1 * C * D + 0.0155 * A^2 \\
 & - 0.182 * B^2 - 0.16075 * C^2 - 0.03575 * D^2
 \end{aligned}$$

where; A- sprouted wheat flour, B- oat, C- sprouted wheat bran and D- guar gum

Table 5 Results of response surface models for WPB

Statistical Parameters	pH	Acidity, %	Probiotic count (ln PC)
Mean	4.443793	0.361724	22.79547
Std. Dev.	0.192294	0.048669	1.136198
C.V., %	4.327252	13.4547	4.984317
PRESS	2.105725	0.080489	42.92384
R-Squared	0.697809	0.781134	0.513828
Adjusted R-Squared	0.395618	0.562268	0.432799
Predicted R-Squared	-0.2292	0.46877	0.326451
Adequate Precision	6.146195	6.838813	9.20421

WPB wheat based probiotic beverage; C.V- Coefficient of variation, PRESS- predicted sum of squares

Table 6 Optimized solutions with predicted and actual experimental values for WPB

Solution no.	Level of ingredients (g)				Desirability	pH		Acidity (% lactic acid)		Probiotic count (log ₁₀ cfu mL ⁻¹)	
	Sprouted wheat flour	Oat	Sprouted wheat bran	Guar gum		Predicted	Exp*	Predicted	Exp*	Predicted	Exp*
1	7.86	5.42	1.42	0.6	0.875787	4.000001	4.18	0.483895	0.46	10.40	10.43
2	7.86	5.4	1.41	0.6	0.87576	4.000001	4.21	0.484363	0.44	10.39	10.26
3	7.86	5.46	1.45	0.6	0.875733	3.999999	4.09	0.483191	0.47	10.41	10.44

WPB wheat based probiotic beverage; Values of responses are mean of three replicates; *Experimental

Effect of ingredients' level on acidity The results of analysis of variance for the response surface quadratic model for acidity are given in Table 3. The R² (coefficient of determination) value of 0.78 for the model *F* value of 3.57 implies that the model is significant for acidity. The non-significant lack of fit of the model is desirable. The response surface graphs for the combined effect of different ingredients on acidity are given in Fig. 2. There is significant effect of sprouted wheat flour and oat on the acidity as acidity increases with the increase in sprouted wheat flour and oat concentration. The second order polynomial model was obtained by model fitting for acidity as the function of sprouted wheat flour, sprouted wheat bran, oat and guar gum as:

$$\begin{aligned} \text{Acidity} = & 0.187041667 + 0.063 * A - 0.035291667 * B \\ & - 0.062666667 * C - 0.427916667 * D \\ & - 0.0040625 * A * B - 0.00625 * A * C \\ & - 0.04375 * A * D - 0.0175 * B * C \\ & - 0.015625 * B * D - 0.1125 * C * D \\ & + 0.00115625 * A^2 + 0.0150625 * B^2 + 0.054 * C^2 \\ & + 1.240625 * D^2 \end{aligned}$$

where; A-sprouted wheat flour, B- oat, C- sprouted wheat bran and D- guar gum

Effect of ingredients' level on probiotic count The response surface linear model was found significant for probiotic count (Table 4) from analysis of variance. The probiotic count of the probiotic beverage samples increased significantly ($p < 0.01$) with the increasing amount of sprouted wheat flour and oat which showed that oat and sprouted wheat flour exerted a beneficial role in maintaining the probiotic bacteria. Thus, these substrates along with *Lactobacillus acidophilus* NCDC-14 can be used for the development of wheat based probiotic beverage. The linear model was obtained by model fitting for natural log (ln) probiotic count as the function of sprouted wheat flour, sprouted wheat bran, oat and guar gum as:

$$\begin{aligned} \text{Ln}(pc) = & 18.5584559 + 0.521932498 * A + 0.56365952 \\ & * B + 0.031422136 * C - 3.030165997 * D \end{aligned}$$

where; A-sprouted wheat flour, B- oat, C- sprouted wheat bran and D- guar gum, pc- probiotic count.

The R² (coefficient of determination) value of 0.514 for the model *F* value of 6.34 implies that the model is significant for the probiotic count, with a non significant lack of fit suggesting that the model is good. The response surface graphs for combined effect of levels of different ingredients on probiotic count are given in Fig. 2.

Validation based upon desirability

The mean values analyzed through Design expert software for pH, acidity and Natural Logarithm (ln) probiotic count were found to be 4.44, 0.36 % and 22.79, respectively (Table 5). The selected formulations (with desirability >0.87) of probiotic beverages were prepared and evaluated for validating the predicted values. Based upon the validation experiments, the formulation with optimized levels for different ingredients viz. sprouted wheat flour, oat, sprouted wheat bran and guar gum as 7.86, 5.42, 1.42 and 0.6 g, respectively per 100 mL of water was found most suitable for preparation of sprouted wheat and oat based probiotic beverage (Table 6).

This probiotic beverage contained 86.81 % moisture and 13.19 % total solids, 1.19 % protein, 0.33 % fat, 0.10 % ash, 0.42 % crude fibre, 11.56 % carbohydrates and 54 kcal calories per 100 mL beverage. The iron and calcium content in this probiotic beverage was 1.45 mg and 15.74 mg per 100 mL, respectively. The probiotic count in this beverage was 10.43 log₁₀ cfu mL⁻¹, which was at par with the suggested level of 7 log₁₀ cfu mL⁻¹ (Gomes and Malcata 1999; Shortt 1999). To provide health benefits, the suggested concentration for probiotic bacteria is 6 log₁₀ cfu mL⁻¹ of a product (Lankaputhra and Shah 1995; Saran et al. 2012).

Conclusion

From the two probiotic strains taken for the study, *L. acidophilus* NCDC-14 was selected for development of sprouted wheat based probiotic beverage as it was more compatible with the substrates. The response surface methodology is a successful tool for optimization. In this study, four ingredients viz. amount of sprouted wheat flour, oat, sprouted wheat bran and guar gum required for maximising the probiotic count were optimized based on response surface methodology. Probiotic count and acidity were significantly affected by the amount of sprouted wheat flour and oat, and maximum probiotic count and acidity were observed at higher concentrations of these ingredients. However, pH was significantly affected by the amount of sprouted wheat flour and other ingredients had a non-significant effect on pH values of the probiotic beverage. The optimum levels of sprouted wheat flour, oat, sprouted wheat bran and guar gum are 7.86, 5.42, 1.42 and 0.6 g respectively per 100 mL of water for the sprouted wheat based probiotic beverage as this formulation resulted in a good probiotic count. Thus, *Lactobacillus acidophilus* NCDC-14 can be used for the development of potentially probiotic wheat based beverage. The wheat based probiotic beverage (100 ml), prepared using the optimized formulation will provide 13.19 % total solids, 1.19 % protein, 0.33 % fat, 0.10 % ash, 0.42 % crude fibre, 1.45 mg iron, calcium 15.74 mg, 11.56 % carbohydrates, 54 kcal calories and $10.43 \log_{10} \text{cfu mL}^{-1}$ probiotic count.

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